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Microbial biosensor for Salmonella using anti-bacterial antibodies isolated from human serum

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ABSTRACT

In this work, we present a novel microbial biosensor for Salmonella based on impedance spectrometry by using isolated antibodies against a specific bacterial strain from human serum. Anti-*Salmonella* (or BL21(DE3)) antibodies were isolated from human serum using *S. enteritidis* (or BL21(DE3)) and the mutant strain ClearColi. After the purification steps, the purification yield of the antibodies was calculated to be 0.2 %. From the FACS analysis, the isolated anti-*Salmonella* antibodies were estimated to have more than 6-fold higher binding affinity for *S. enteritidis* compared to antibodies against other kinds of Gram-negative bacterial strains, including HB101, ClearColi, JM110, DH5α, and BL21(DE3). Finally, the anti-*Salmonella* antibodies isolated herein were used for bacterial detection using electrochemical biosensors based on impedance spectrometry and the R_{ct} value of the antibodies was estimated for *S. enteritidis* from the Nyquist plot. The limit of detection of the isolated anti-*Salmonella* antibodies was estimated to be 1.0×10^3 cells/mL for *S. enteritidis* and 1.0×10^6 cells/mL for BL21(DE3), respectively.

1. Introduction

Microbial biosensors have been developed for the detection of bacteria without enrichment steps based on various kinds of transducers such as electrochemical, fluorescent, mass sensitive, and chemiluminescent detectors [1–13]. For the sensitive detection of specific bacterial strains in complex mixtures, the specific interaction between antigens and antibodies has been most frequently used. Such a detection of bacterial strains using antibodies requires their binding to target antigens on the outer membrane of bacterial cells. However, the production of such antibodies for the binding to specific bacterial strains is known to be very difficult because the outer membrane of target bacteria contain harmful enterotoxins [14–16] For example, gram-negative bacteria have lipopolysaccharides (LPS) and gram-positive bacteria have lipoteichoic acids (LTA) on the outer membranes of their cells. Such enterotoxins are known to be strong pyrogens and typically cause fever in animals when administered via intravenous or intraperitoneal injection [17]. Such enterotoxins are known to be very harmful to antibody-producing animals; the mass-production of antibodies against such enterotoxins is nearly impossible [18–22]. Recently, a novel isolation method was reported for antibodies against specific bacterial strains from human serum using a target bacterial strain and a mutant *E. coli* strain without LPS (ClearColi) [15,18,23]. As shown in Fig. 1(a), the target bacteria were incubated with human serum and then, the bound proteins were dissociated by acid treatment. The prepared dissociated protein solution contained antibodies against the target bacteria, as well as other proteins that could bind to the outer membrane of gram-negative bacteria. To isolate antibodies against the target bacteria, the dissociated protein solution was incubated with the modified *E. coli* strain without LPS (ClearColi) to remove the non-specifically bound proteins.

In this work, anti-*Salmonella* (or BL21(DE3)) antibodies were isolated from human serum using *S. enteritidis* (or BL21(DE3)) and the mutant strain ClearColi. After the purification steps, the purification yield was calculated and the binding affinity was analyzed from the FACS analysis and compared to that of antibodies against other gram-negative bacterial strains, including HB101, ClearColi, JM110, DH5α, and BL21(DE3). Finally, the isolated anti-*Salmonella* antibodies were used for bacterial detection using electrochemical biosensors based on impedance spectrometry [24].

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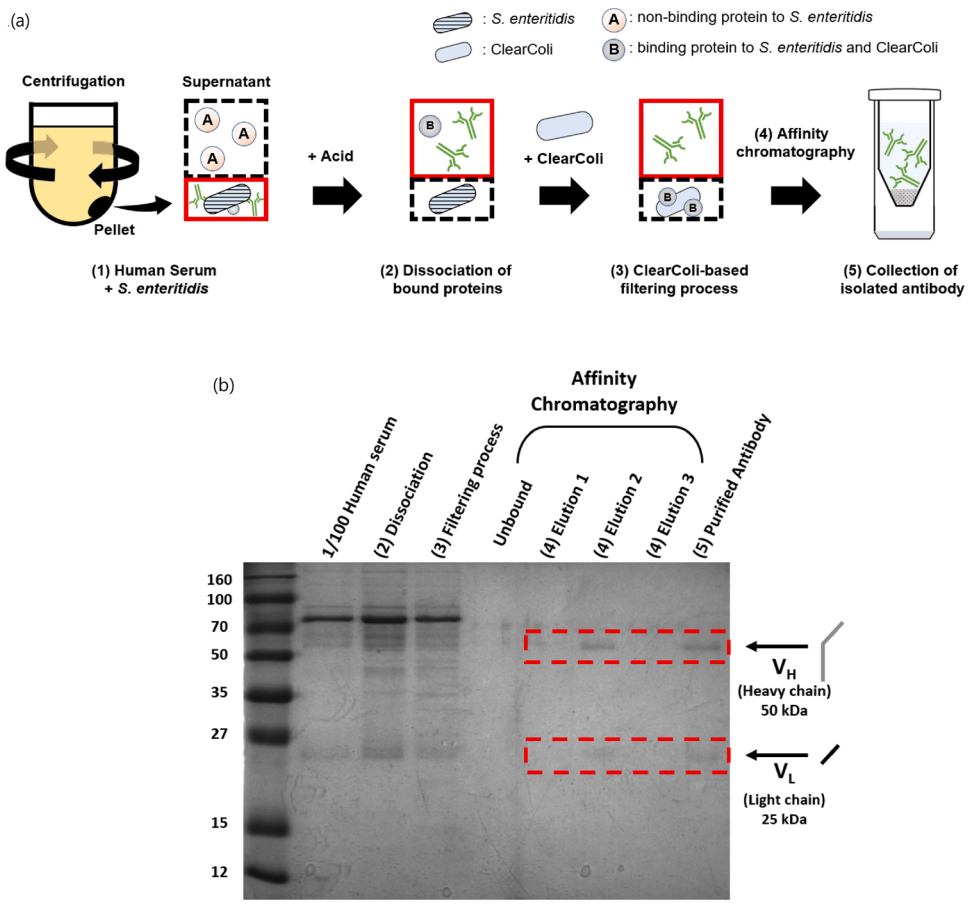

Fig. 1. Isolation of anti-*Salmonella* antibodies from human serum.
(a) Steps for isolating anti-*Salmonella* antibodies using *E. coli* and ClearColi.
(b) SDS-PAGE analysis of proteins from each isolation step.

2. Experimental

2.1. Materials

Amicon centrifugal filter, potassium ferricyanide, glycine, rhodamine B, human serum, bovine serum albumin (BSA), Tween®-20, YT medium, and antibiotics (carbenicillin, kanamycin, and chloramphenicol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Luria-Bertani (LB) broth was purchased from Duchefa (Haarlem, The Netherlands). Phosphate-buffered saline (PBS) was purchased from Curebio (Seoul, Korea). Fluorescein isothiocyanate (FITC)-conjugated anti-human IgG (anti-human IgG) and Cyanine 5 (Cy5)-conjugated anti-human IgG were purchased from Abcam Co. (Cambridge, UK). The Ag/AgCl reference electrode was purchased from Warner Instruments, LLC (Hamden, CO, USA).

2.2. Separation of anti-LPS antibodies

S. enteritidis (or BL21(DE3)) and ClearColi™ BL21(DE3) were used to separate polyclonal anti-*Salmonella* (or BL21(DE3)) antibodies from human serum [15]. BL21(DE3) is a strain modified from BL21 that contains the coding sequence of T7 RNA polymerase, which is required for the protein expression system based on the T7 promotor [25]. *S. enteritidis* and *E. coli* strains BL21(DE3), ClearColi, HB101, DH5α, and JM110 were cultured at 37 °C in LB broth containing 10 μM ethylenediaminetetraacetic acid (EDTA) for 16 h. The cultured *S. enteritidis* (or *E. coli* BL21(DE3)) cells (1 mL) were harvested by centrifugation at 7000 *g* for three min and washed three times with PBS (1 mL). The cells were resuspended in PBS and the optical density was measured at a wavelength of 600 nm (OD_{600}) using a microplate reader (Versamax, Molecular Devices, San Jose, CA, USA); the final concentration was adjusted to be $OD_{600} = 1.0$, which corresponded to 5×10^8 cells/mL [26–29]. The anti-*Salmonella* (or BL21(DE3)) antibodies in the human serum were separated using the following steps, as shown in Fig. 1(a): (1) Human serum (1 mL) was incubated with *S. enteritidis* (or BL21 (DE3)) cells (10^7 cells) for 1 h under mild shaking conditions (20 rpm) using a wheel-rotating mixer (RT-10 from Daehan Scientific Co, Wonju, Korea); (2) The bound proteins (including anti-*Salmonella* (or BL21 (DE3)) antibodies) were dissociated from *S. enteritidis* (or BL21(DE3)) by treatment with 1 mL of 0.1 M glycine−HCl buffer (pH 2.7) for 10 s. Then, the reaction was neutralized by the addition of 55 μL of 1 M Tris−HCl buffer (pH 10.5); (3) The dissociated proteins were added to 10^7 cells of ClearColi to remove other unwanted proteins. This ClearColi-based filtering process was repeated three times; (4) The remaining antibody fraction was separated using a protein-A column. First, the dissociated proteins were loaded onto the protein-A column. Then, the column was washed with 10 mL of PBS to remove the unbound proteins. The bound anti-LPS antibodies were eluted by loading 3 mL of 0.1 M glycine−HCl buffer (pH 2.7) onto the column. The eluted polyclonal antibodies were directly collected in a microcentrifuge tube containing 165 μL of 1 M Tris−HCl buffer (pH 10.5). Finally, the polyclonal antibodies were concentrated by Amicon centrifugal filtration (molecular weight cutoff: 3000 Da). The yield of polyclonal antibodies was defined as the amount of protein separated from unit volume (1 mL) of human serum. The amount of protein was calculated using a bicinchoninic acid (BCA) assay kit (Thermo Fisher Scientific, Waltham, MA, USA).

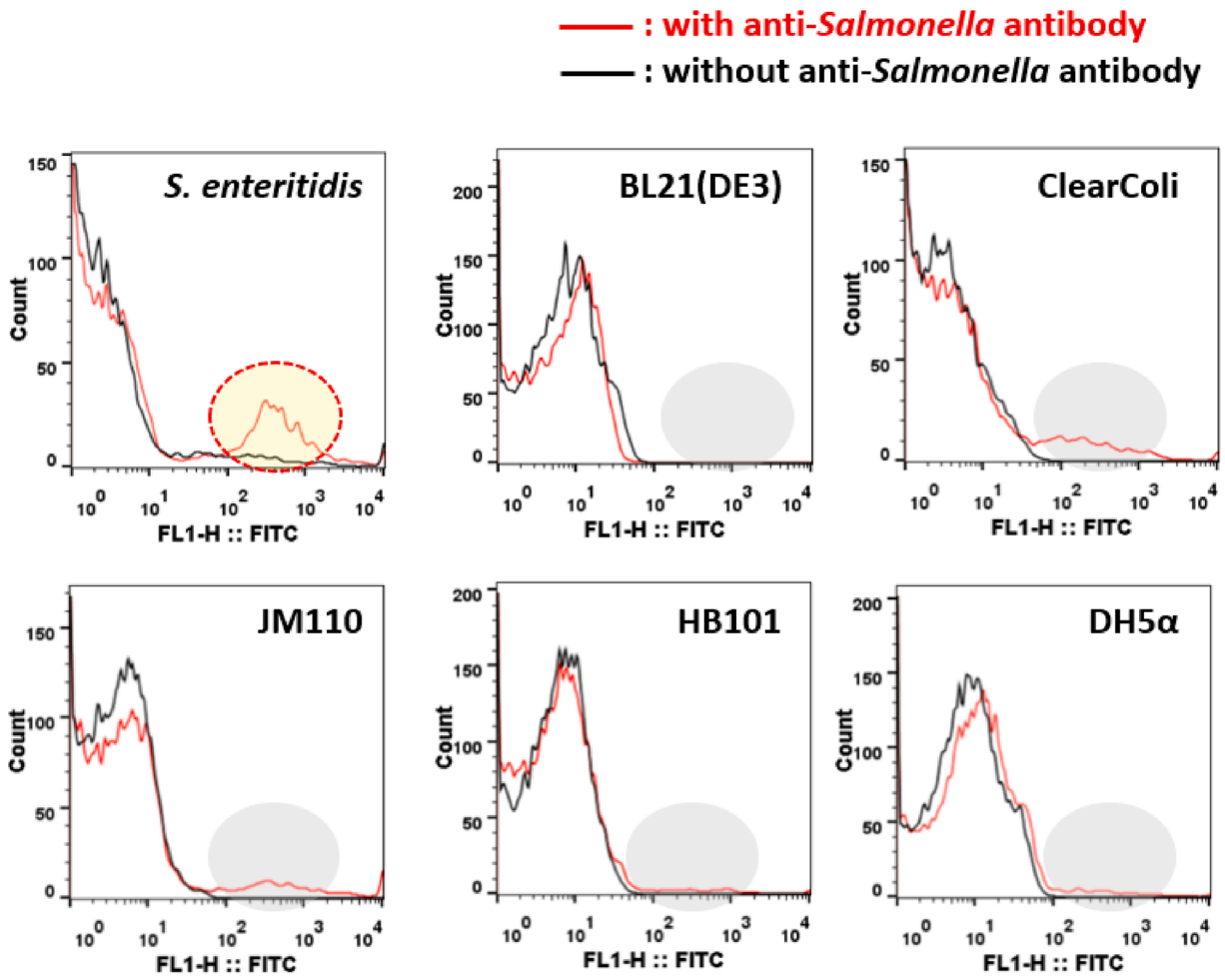

Fig. 2. Strain specificity of the antibodies isolated from human serum. FACS analysis of the binding of the antibodies to cells of different bacterial strains.

2.3. Assays carried out with the isolated antibodies

The measurement of the binding properties of the isolated antibodies towards *S. enteritidis*, ClearColi, HB101, JM110, DH5α, and BL21(DE3) was carried out by FACS analysis with a FACScaliber instrument from Becton-Dickinson Co. (Franklin Lakes, NJ, USA) [30,31]. FACS analysis was carried out by sequentially reacting the isolated anti-*Salmonella* antibodies at the concentration of OD_{600} = 1.0 for 1 h. After washing thrice with 0.1 % Tween®-20 by centrifugation, treatment with secondary anti-human antibodies labeled with fluorescein at the concentration of 10 μg/mL was performed for 1 h. FACS analysis was carried out using a bacterial solution at the concentration of 10^7 cells/mL and the fluorescence count (Y-axis) was measured against the intensity of fluorescence (X-axis) in the logarithmic scale.

The anti-*Salmonella* antibodies were separately incubated with three bacterial strains, *S. enteritidis*, BL21(DE3), and ClearColi at the concentration of OD_{600} = 1.0 for 1 h. After washing by centrifugation, these three bacterial strains were incubated with secondary antibodies against anti-human IgG labeled with Cy5 (λ_{ex} =649 nm, λ_{em} =670 nm) at the concentration of 10 μg/mL for 1 h. After washing by centrifugation, the bacterial strains were immobilized on a glass slide by dropping 2 μL of cells onto the slide. After drying at room temperature, the intensity of fluorescence at each spot was measured using a DNA scanner from Molecular Devices Co (San Jose, CA, USA).

Impedance spectroscopy was performed using a three-electrode system and a potentiostat from IVIUM Technologies (Eindhoven, Netherlands) [32,33]. A circular gold electrode with a diameter of 6 mm and a thickness of 100 nm on a SiO_2 wafer was used as the working electrode. The isolated antibodies were immobilized on the electrode at a concentration of 20 μg/mL for 2 h. Incubation with BSA (1 mg/mL) for 1 h was performed to block the electrode; this inhibits non-specific binding. Bacterial solution (100 μL) was added to the electrode and incubated for 30 min. Each detection was performed after a washing step with PBS. Impedance spectroscopy was measured in the frequency range of 0.1 Hz to 1 MHz at 10 mV amplitude versus the Ag/AgCl reference electrode at an amplitude of 50 mV in 50 mM potassium ferricyanide. The measured impedance was analyzed using a Randles equivalent circuit model, which consisted of a parallel combination of double layer capacitance (C_{dl}) and charge transfer resistance (R_{ct}) with a series medium resistance (R_s).

3. Results and discussion

3.1. Isolation and immobilization of anti-LPS antibodies

Anti-*Salmonella* (or BL21(DE3)) antibodies were isolated from human serum using *S. enteritidis* (or BL21(DE3)) and the mutant ClearColi strain (Fig. 1(a)). Human serum was first incubated with *S. enteritidis* (or BL21(DE3)) and then, the bound protein on the *S. enteritidis* (or BL21(DE3)) cells was dissociated by treatment with an acid solution (0.1 M glycine−HCl, pH 2.7). The dissociated protein solution contained anti-*Salmonella* (or BL21(DE3)) antibodies, as well as other proteins bound to the outer membrane. To isolate only the anti-*Salmonella* (or BL21(DE3)) antibodies from the dissociated protein solution, ClearColi was incubated with the solution containing the dissociated proteins. Because the proteins bound to ClearColi showed affinity towards the

Table 1
Summary of FACS analysis for different *E. coli* strains.

	S. enteritidis	BL21(DE3)	ClearColi	JM110	HB101	DH5α
Fluorescence signal with anti-S. antibodies (A)	15.5	5.7	4.0	4.5	4.9	9.1
Fluorescence signal without anti-S. antibodies (B)	2.4	5.1	2.5	3.3	4.9	7.0
Relative ratio of fluorescence signal (A/B, %)	651.3 %	111.8 %	162.4 %	135.4 %	99.4 %	129.6 %

outer membrane of *S. enteritidis* (or BL21(DE3)) as well as ClearColi, the anti-*Salmonella* (or BL21(DE3)) antibodies were not bound to ClearColi and they remained in the solution. Finally, the anti-*Salmonella* (or BL21 (DE3)) antibodies were isolated using a protein-A column.

The isolation of anti-*Salmonella* (or BL21(DE3)) antibodies was carried out through the steps described above. The protein fraction of each isolation step was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1(b). As the antibodies are composed of heavy chains (V_H, 55 kDa) and light chains (V_L, 25 kDa), antibodies were usually observed to show bands for V_H, V_L, and V_H+V_L (75 kDa) on the SDS-polyacrylamide gel. In this work, the initial protein concentration of human serum was estimated to be 63.6 mg/mL. The concentration of protein separated after the repeated binding to ClearColi was estimated to be 78.6, 112.9, 81.6, and 47.3 μg/mL (1 mL for each elution) for the 1st, 2nd, 3rd, and 4th elution samples, respectively. After the purification step with protein-A, the amount of anti-*Salmonella* antibodies isolated from human serum (1 mL) was determined to be 123.5 μg from the total protein (63.6 mg); thus, the purification yield was calculated to be 0.2 %.

3.2. Properties of immobilized anti-LPS antibodies

The binding properties of the isolated anti-*Salmonella* antibodies were characterized based on their: (1) binding specificity to *S. enteritidis* among other strains using FACS analysis and (2) binding affinity constants towards *S. enteritidis*. First, the binding properties of the isolated anti-*Salmonella* antibodies towards other strains of bacteria such as HB101, ClearColi, JM110, DH5α, and BL21(DE3) were estimated using FACS analysis. FACS analysis was carried out by sequentially allowing the isolated anti-*Salmonella* antibodies and secondary anti-human antibodies labeled with fluorescein (λ_{ex} =488 nm, λ_{em} =508 nm) to react (Supplement Fig. S1). Bacteria with no affinity to the isolated antibodies were expected to present no significant fluorescence. FACS analysis was performed to measure the intensity of fluorescence (X-axis) and fluorescence counts (Y-axis). As shown in Fig. 2, *S. enteritidis* showed significantly different fluorescence intensity before and after the treatment with the isolated anti-*Salmonella* antibodies, which represents the specific binding of the antibodies to *S. enteritidis*. Other bacterial strains showed no significant change in fluorescence intensity before and after treatment with the isolated anti-*Salmonella* antibodies, which represented no significant binding by the antibodies. The binding affinity to each strain was calculated from the fluorescence intensity difference before and after treatment with the anti-*Salmonella* antibodies (summarized in Table 1). From this calculation, the relative affinity of the isolated anti-LPS antibodies towards *S. enteritidis* was estimated to be 651.3 %, which was considered to be a far higher relative affinity than that towards other strains such as BL21(DE3) (109.9 %), ClearColi (162.4 %), JM110 (135.4 %), HB101 (99.4 %), and DH5α (129.6 %) as shown in Fig. 2. Thus, these results showed that the isolated anti-*Salmonella* antibodies had significantly different binding affinities to *S. enteritidis* and the other strains, and that they could also be used to discriminate between different bacterial strains.

Next, the binding sensitivity of the isolated anti-*Salmonella* antibodies was compared using *S. enteritidis*, BL21(DE3), and ClearColi (negative control). The anti-*Salmonella* antibodies were separately

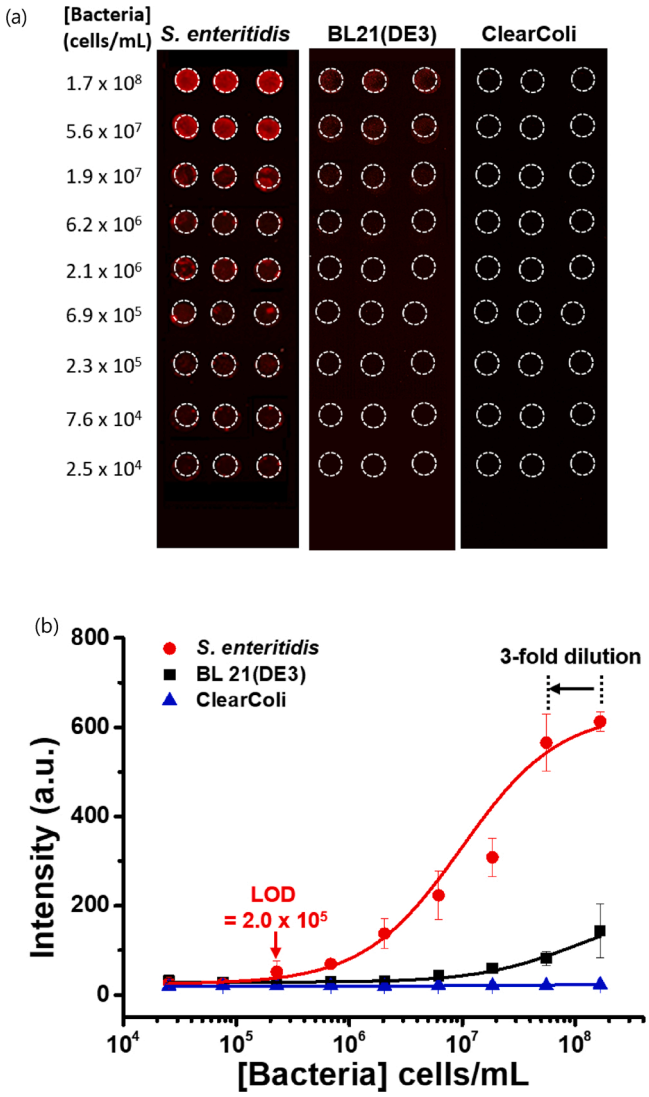

Fig. 3. Characterization of isolated anti-*Salmonella* antibodies from human serum.
(a) Fluorescence image of the binding of *E. coli* to anti-*Salmonella* antibodies.
(b) Analysis of the binding of *E. coli* to anti-*S. enteritidis* antibodies.

incubated with the three bacterial strains in the concentration range of $1.7 \times 10^8 - 2.5 \times 10^4$ cells/mL. After washing by centrifugation, these three bacterial strains were incubated with secondary antibodies against anti-human IgG labeled with Cy5 (λ_{ex} =649 nm, λ_{em} =670 nm). After washing by centrifugation, the three bacterial strains were immobilized on a glass slide, and then, the intensity of fluorescence at each spot was estimated using a DNA scanner. As shown in Fig. 3(a), the fluorescence image shows a significant difference in the binding affinity of the anti-*Salmonella* antibodies to *S. enteritidis*, BL21(DE3), and ClearColi. These results showed that anti-*Salmonella* antibodies could be used for the

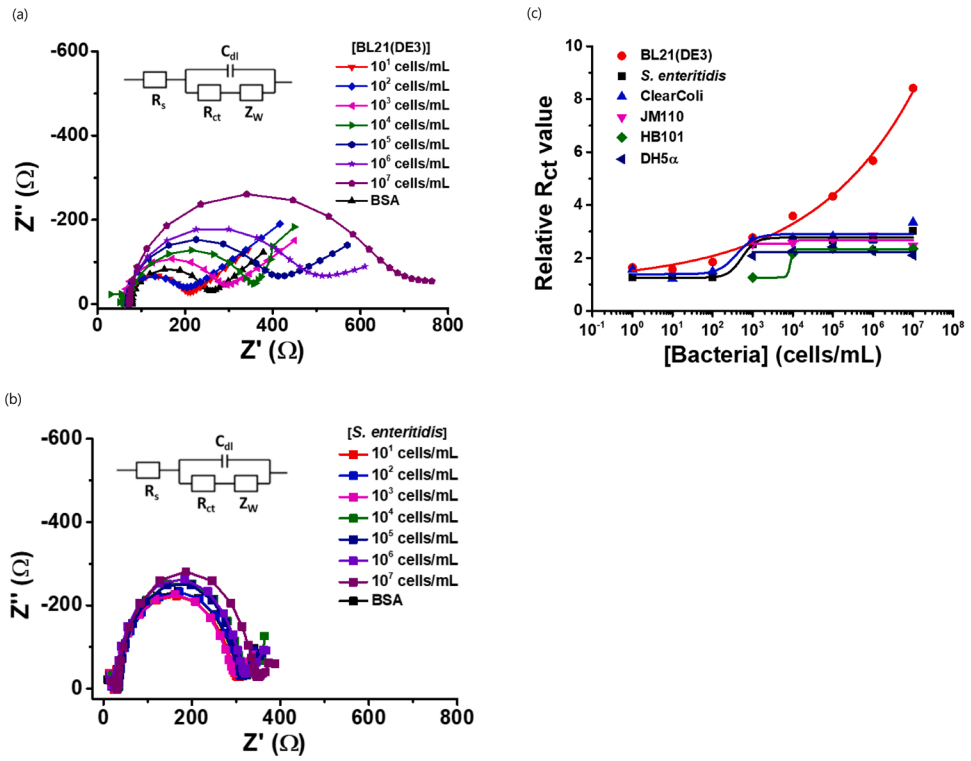

Fig. 4. Impedance immunoassay using anti-BL21(DE3) antibodies from human serum.
(a) Nyquist plot of anti-BL21(DE3) antibodies against BL21(DE3).
(b) Nyquist plot of anti-BL21(DE3) antibodies against *S. enteritidis*.
(c) R_{ct} analysis for BL-21(DE3) detection using anti-BL21(DE3) antibodies isolated from human serum.

selective detection of *S. enteritidis* from BL21(DE3) and ClearColi. As shown in the binding curves in Fig. 3(b), the isolated anti-*Salmonella* antibodies show significantly higher sensitivity towards *S. enteritidis* than towards the other bacterial strains BL21(DE3) and ClearColi at a concentration range higher than 1.0×10^5 cells/mL. The limit of detection of the isolated anti-*Salmonella* antibodies was estimated to be 2.0×10^5 cells/mL for *S. enteritidis* and 1.4×10^7 cells/mL for BL21 (DE3). The LOD value was estimated to be the average intensity of negative sample with three-times of standard deviation (average value + 3σ, n = 3) [34]. These results showed that the isolated anti-*Salmonella* antibodies could effectively bind to *S. enteritidis*, compared to other bacterial strains.

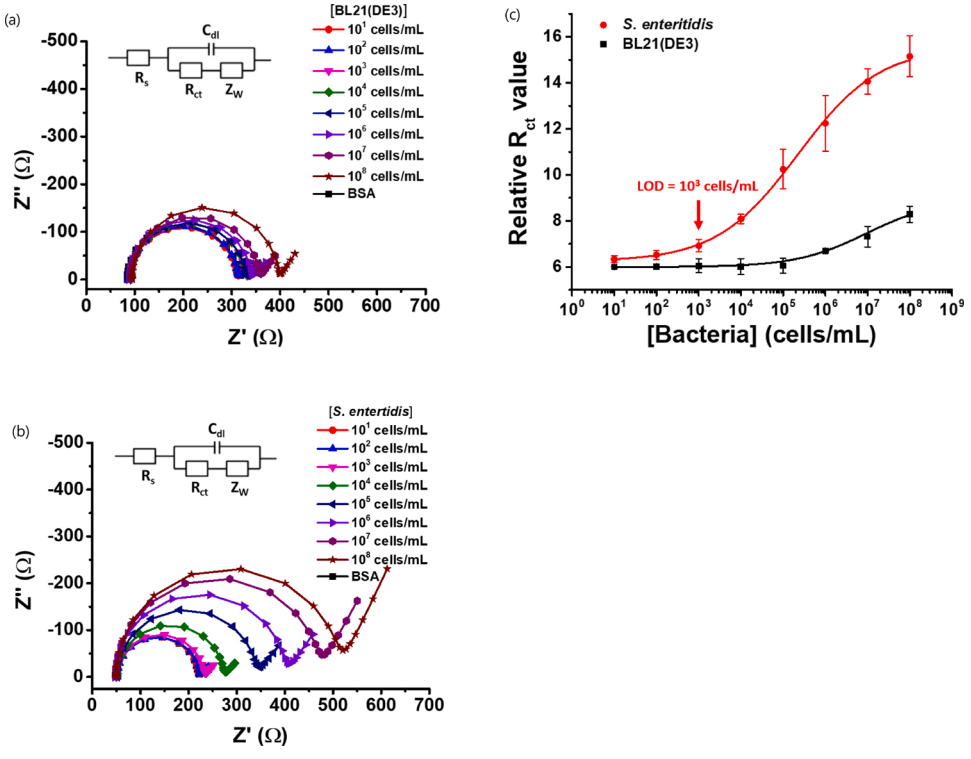

Fig. 5. Impedance immunoassay using anti-*Salmonella* antibodies from human serum.
(a) Nyquist plot of anti-*Salmonella* antibodies against BL21(DE3).
(b) Nyquist plot of anti-*Salmonella* antibodies against *S. enteritidis*.
(c) R_{ct} analysis for *S. enteritidis* detection using anti-*Salmonella* antibodies isolated from human serum.

3.3. Application of isolated antibodies for bacterial detection

The binding properties of the isolated anti-BL21(DE3) antibodies were used for bacterial detection with impedance spectrometry using anti-BL21(DE3) antibodies and anti-*Salmonella* antibodies. First, the isolated anti-BL21(DE3) antibodies were used for bacterial detection using electrochemical biosensors based on impedance spectrometry. The impedance spectrometry was carried out in the frequency range of 10^{-1} – 10^{6} Hz at a 10 mV potential against the Ag/AgCl reference electrode using a gold electrode with an area of 28 mm^2 as a working electrode. The impedance spectra of the Nyquist plot were measured for BL21 (DE3) and *S. enteritidis* in the concentration range of 1.0×10^{1} – 1.0×10^{7} for cells/mL (BL21(DE3), *S. enteritidis*, ClearColi) and 1.0×10^{3} – 1.0×10^{7} for cells/mL (JM110, DH5α, HB101) (Fig. 4(a) and (b)). From the Nyquist plot (Fig. 4(a) and (b)), the signal of the charge transfer resistance (R_{ct}) was taken from the fitting based on the Randles equivalent circuit model, which consisted of a parallel combination of double layer capacitance (C_{dl}) and charge transfer resistance (R_{ct}) with a series medium resistance (R_s) [35]. As shown in Fig. 4(c), the R_{ct} value is observed to be quantitatively increased for the BL21 samples as the bacterial concentration increased. The relative charge transfer resistance (R_{ct}) was calculated by normalizing the change in R_{ct} after the adsorption of analyte on the electrode with the initial R_{ct} value of electrode. The calculation was performed as following equation: Relative R_{ct} value = $[R_{ct} - R_{ct0}] / R_{ct0}$, where R_{ct} represented R_{ct} value after the adsorption of analyte on the electrode and R_{ct0} represented the initial R_{ct} value of electrode. These data showed that the active area of the working electrode decreased and the R_{ct} value increased following the binding of bacteria to the immobilized anti-BL21(DE3) antibodies. Similar to the FACS analysis, the R_{ct} value for BL21(DE3) was significantly higher than that for the other bacterial strains because the isolated anti-BL21(DE3) antibodies could specifically and quantitatively bind to BL21 compared to the other bacterial strains. The detectable range of the isolated anti-BL21(DE3) antibodies was up to 10^{3} cells/mL for BL21(DE3). These results showed that the isolated anti-BL21(DE3) antibodies could be used for the specific detection of BL21 using fluorescence and impedance spectrometry-based immunoassays.

As the next step, the isolated anti-*Salmonella* antibodies were used for bacterial detection using electrochemical biosensors and impedance spectrometry. The impedance spectrometry was carried out as described above. The impedance spectra of the Nyquist plot were measured for BL21(DE3) and *S. enteritidis* in the concentration range of 1.0×10^{1} – 1.0×10^{8} cells/mL (Fig. 5(a) and (b). From the Nyquist plot (Fig. 5(a) and (b)), the signal of the charge transfer resistance (R_{ct}) was taken from the fitting based on the Randles equivalent circuit model. As shown in Fig. 5(c), the R_{ct} value is observed to be quantitatively increased for the *S. enteritidis* samples as the bacterial concentration increased. As previously mentioned, the relative charge transfer resistance (R_{ct}) was calculated by normalizing the change in R_{ct} after the adsorption of analyte on the electrode with the initial R_{ct} value of electrode. The calculation was performed as following equation: Relative R_{ct} value = $[R_{ct} - R_{ct0}] / R_{ct0}$, where R_{ct} represented R_{ct} value after the adsorption of analyte on the electrode and R_{ct0} represented the initial R_{ct} value of electrode. These data showed that the active area of the working electrode decreased and the R_{ct} value increased following the binding of bacteria to the immobilized anti-*Salmonella* antibodies. Similar to the FACS analysis, the R_{ct} value for *S. enteritidis* was significantly higher than that of the other bacterial strains because the isolated anti-*Salmonella* antibodies could specifically and quantitatively bind to *S. enteritidis*. The limit of detection of the isolated anti-*Salmonella* antibodies was estimated to be 1.0×10^{3} cells/mL for *S. enteritidis* and 1.0×10^{6} cells/mL for BL21(DE3). These results showed that the isolated anti-*Salmonella* antibodies could be used for the specific detection of BL21 using fluorescence and impedance spectrometry-based immunoassays.

4. Conclusions

Anti-*Salmonella* (or BL21(DE3)) antibodies were isolated from human serum using *S. enteritidis* (or BL21(DE3)) and the mutant strain ClearColi. After the purification step with protein-A, the amount of isolated anti-*Salmonella* antibodies from human serum (1 mL) was determined to be 123.5 μg from total protein (63.6 mg); thus, the purification yield was calculated to be 0.2 %. The binding properties of the isolated anti-*Salmonella* antibodies were estimated for other strains of bacteria such as HB101, ClearColi, JM110, DH5α, and BL21(DE3) using FACS analysis. The isolated anti-*Salmonella* antibodies had a significantly higher binding affinity towards *S. enteritidis* (more than 6-fold higher) than that towards the other bacterial strains tested. The limit of detection of the isolated anti-*Salmonella* antibodies was estimated to be 2.0×10^{5} cells/mL for *S. enteritidis* and 1.4×10^{7} cells/mL for BL21 (DE3). These results showed that the isolated anti-*Salmonella* antibodies could effectively bind to *S. enteritidis*, compared to the other bacterial strains. The isolated anti-*Salmonella* antibodies were used for bacterial detection using electrochemical biosensors based on impedance spectrometry; the R_{ct} value from the Nyquist plot was observed to gradually increase for the *S. enteritidis* samples as the bacterial concentration increased. The limit of detection of the isolated anti-*Salmonella* antibodies from the impedance spectrometry was estimated to be 1.0×10^{3} cells/mL for *S. enteritidis* and 1.0×10^{6} cells/mL for BL21(DE3). These results showed that the isolated anti-*Salmonella* antibodies could be used for the specific detection of *S. enteritidis* using fluorescence and impedance spectrometry-based immunoassays.

Author agreement

The following authors agree to submit the manuscript titled "Microbial biosensor for Salmonella using anti-bacterial antibodies isolated from human serum" to publish in Enzyme and Microbial Technology.

CRediT authorship contribution statement

Jun-Hee Park: Data curation, Writing - original draft. **Ji-Hong Bong:** Data curation, Writing - original draft. **Jaeyong Jung:** Data curation. **Jeong Soo Sung:** Data curation. **Ga-Yeon Lee:** Data curation. **Min-Jung Kang:** . **Jae-Chul Pyun:** Supervision, Funding acquisition, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.enzmictec.2020.109721.

References

[1] S.R. Chinnadayyala, J. Park, H.T.N. Le, M. Santhosh, A.N. Kadam, S. Cho, Recent advances in microfluidic paper-based electrochemiluminescence analytical devices for point-of-care testing applications, Biosens. Bioelectron. 126 (2019) 68–81, https://doi.org/10.1016/j.bios.2018.10.038.

[2] P.B. Luppa, L.J. Sokoll, D.W. Chan, Immunosensors - Principles and applications to clinical chemistry, Clin. Chim. Acta 314 (2001) 1–26, https://doi.org/10.1016/S0009-8981(01)00629-5.

[3] H.R. Kim, J.H. Bong, J. Jung, J.S. Sung, M.J. Kang, J.G. Park, J.C. Pyun, An on-chip chemiluminescent immunoassay for bacterial detection using in situ-synthesized cadmium sulfide nanowires with passivation layers, Biochip J. 14 (2020) 268–278, https://doi.org/10.1007/s13206-020-4305-1.

[4] S. Mishra, T.R. Rautray, Silver-incorporated hydroxyapatite–albumin microspheres with bactericidal effects, J. Korean Ceram. Soc. 57 (2020) 175–183, https://doi.org/10.1007/s43207-020-00018-z.
[5] P. Wiecinska, A. Zurawska, P. Falkowski, D.Y. Jeong, M. Szafran, Sweet ceramics: how saccharide-based compounds have changed colloidal processing of ceramic materials, J. Korean Ceram. Soc. 57 (2020) 231–245, https://doi.org/10.1007/s43207-020-00036-x.
[6] G.Y. Lee, J.H. Bong, J.Y. Kim, G. Yoo, M. Park, M.J. Kang, J. Jose, J.C. Pyun, Thermophoretic diagnosis of autoimmune diseases based on Escherichia coli with autodisplayed autoantigens, Anal. Chim. Acta 1055 (2019) 106–114, https://doi.org/10.1016/j.aca.2018.12.038.
[7] J.K. Lee, M. Gu-Yoo, J. Park, M.J. Jose, J.C. Kang, Pyun, Electrochemical ELISA based on Escherichia coli with autodisplayed Z-domains, Sensors Actuators, B Chem. 175 (2012) 46–52, https://doi.org/10.1016/j.snb.2011.11.064.
[8] Z. Shen, M. Huang, C. Xiao, Y. Zhang, X. Zeng, P.G. Wang, Nonlabeled quartz crystal microbalance biosensor for bacterial detection using carbohydrate and lectin recognitions, Anal. Chem. 79 (2007) 2312–2319, https://doi.org/10.1021/ac061986j.
[9] A. Fulgione, M. Cimafonte, B. Della Ventura, M. Iannaccone, C. Ambrosino, F. Capuano, Y.T.R. Proroga, R. Velotta, R. Capparelli, QCM-based immunosensor for rapid detection of Salmonella typhimurium in food, Sci. Rep. 8 (2018) 1–8, https://doi.org/10.1038/s41598-018-34285-y.
[10] L. Guo, X. Zhou, D.H. Kim, Facile fabrication of distance-tunable Au-nanorod chips for single-nanoparticle plasmonic biosensors, Biosens. Bioelectron. 26 (2011) 2246–2251, https://doi.org/10.1016/j.bios.2010.09.042.
[11] J.C. Pyun, H. Beutel, J.U. Meyer, H.H. Ruf, Development of a biosensor for E. Coli based on a flexural plate wave (FPW) transducer, Biosens. Bioelectron. 13 (1998) 839–845, https://doi.org/10.1016/S0956-5663(98)00050-5.
[12] J.W. Lee, V.D. Nguyen, T.S. Seo, Paper-based molecular diagnostics for the amplification and detection of pathogenic Bacteria from human whole blood and milk without a sample preparation step, Biochip J. 13 (2019) 243–250, https://doi.org/10.1007/s13206-019-3310-8.
[13] C. Park, M. Kong, J.H. Lee, S. Ryu, S. Park, Detection of Bacillus Cereus Using bioluminescence assay with cell wall-binding domain conjugated magnetic nanoparticles, Biochip J. 12 (2018) 287–293, https://doi.org/10.1007/s13206-018-2408-8.
[14] S. Joo, J. Lim, Y. Nam, Design and fabrication of miniaturized neuronal circuits on microelectrode arrays using agarose hydrogel micro-molding technique, Biochip J. 12 (2018) 193–201, https://doi.org/10.1007/s13206-018-2308-y.
[15] J.H. Bong, J. Kim, G.Y. Lee, J.H. Park, T.H. Kim, M.J. Kang, J.C. Pyun, Fluorescence immunoassay of E. Coli using anti-lipopolysaccharide antibodies isolated from human serum, Biosens. Bioelectron. 126 (2019) 518–528, https://doi.org/10.1016/j.bios.2018.10.036.
[16] R. Praharaj, S. Mishra, T.R. Rautray, The structural and bioactive behaviour of strontium-doped titanium dioxide nanorods, J. Korean Ceram. Soc. 57 (2020) 271–280, https://doi.org/10.1007/s43207-020-00027-y.
[17] J. Yang, S.S. Woo, Y.H. Ryu, C.H. Yun, M.H. Cho, G.E. Rhie, B.S. Kim, H.B. Oh, S. H. Han, Bacillus anthracis lethal toxin attenuates lipoteichoic acid-induced maturation and activation of dendritic cells through a unique mechanism, Mol. Immunol. (2009), https://doi.org/10.1016/j.molimm.2009.08.005.
[18] M.A. Apicella, M.A.J.J. Westerink, S.A. Morse, H. Schneider, P.A. Rice, J. M. Griffiss, Bactericidal antibody response of normal human serum to the lipooligosaccharide of Neisseria gonorrhoeae, J. Infect. Dis. 153 (1986) 520–526, https://doi.org/10.1093/infdis/153.3.520.
[19] W.C. Bogard, D.L. Dunn, K. Abernethy, C. Kilgarriff, P.C. Kung, Isolation and characterization of murine monoclonal antibodies specific for gram-negative bacterial lipopolysaccharide: association of cross-genus reactivity with lipid A specificity, Infect. Immun. 55 (1987) 899–908.
[20] P.A. Dale, D.P. McQuillen, S. Gulati, P.A. Rice, Human vaccination with Escherichia coli J5 mutant induces cross-reactive bactericidal antibody against Neisseria gonorrhoeae lipooligosaccharide, J. Infect. Dis. 166 (1992) 316–325, https://doi.org/10.1093/infdis/166.2.316.
[21] C.M. O'Shaughnessy, F. Micoli, M. Gavini, M. Goodall, M. Cobbold, A. Saul, C. A. MacLennan, Purification of antibodies to O antigen of Salmonella typhimurium from human serum by affinity chromatography, J. Immunol. Methods 387 (2013) 199–210, https://doi.org/10.1016/j.jim.2012.10.015.
[22] S. Patris, M. Vandeput, J.M. Kauffmann, Antibodies as target for affinity biosensors, TrAC - Trends Anal. Chem. 79 (2016) 239–246, https://doi.org/10.1016/j.trac.2015.12.005.
[23] J.H. Bong, T.H. Kim, J. Jung, S.J. Lee, J.S. Sung, C.K. Lee, M.J. Kang, H.O. Kim, J. C. Pyun, Pig sera-derived Anti-SARS-CoV-2 antibodies in surface plasmon resonance biosensors, Biochip J. 14 (2020) 358–368, https://doi.org/10.1007/s13206-020-4404-z.
[24] H.T. Ngoc Le, J. Kim, J. Park, S. Cho, A review of electrical impedance characterization of cells for label-free and real-time assays, Biochip J. 13 (2019) 295–305, https://doi.org/10.1007/s13206-019-3401-6.
[25] F.W. Studier, B.A. Moffatt, Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes, J. Mol. Biol. 189 (1986) 113–130, https://doi.org/10.1016/0022-2836(86)90385-2.
[26] K.D. Mandakhalikar, J.N. Rahmat, E. Chiong, K.G. Neoh, L. Shen, P.A. Tambyah, Extraction and quantification of biofilm bacteria: method optimized for urinary catheters, Sci. Rep. 8 (2018) 1–9, https://doi.org/10.1038/s41598-018-26342-3.
[27] Y. Raymond, C.P. Champagne, The use of flow cytometry to accurately ascertain total and viable counts of Lactobacillus rhamnosus in chocolate, Food Microbiol. 46 (2015) 176–183, https://doi.org/10.1016/j.fm.2014.07.002.
[28] B. Volkmer, M. Heinemann, Condition-Dependent cell volume and concentration of Escherichia coli to facilitate data conversion for systems biology modeling, PLoS One 6 (2011) 1–6, https://doi.org/10.1371/journal.pone.0023126.
[29] J.H. Bong, G. Yoo, M. Park, M.J. Kang, J. Jose, J.C. Pyun, Ultrasonic isolation of the outer membrane of Escherichia coli with autodisplayed Z-domains, Enzyme Microb. Technol. 66 (2014) 42–47, https://doi.org/10.1016/j.enzmictec.2014.08.006.
[30] M. Park, J.H. Bong, Y.W. Chang, G. Yoo, J. Jose, M.J. Kang, J.C. Pyun, FACS-based immunoassay of troponin-I using E. Coli cells with autodisplayed Z-domains, Anal. Methods 6 (2014) 1700–1708, https://doi.org/10.1039/c3ay42206b.
[31] J. Jose, J.W. Chung, B.J. Jeon, R.M. Maas, C.H. Nam, J.C. Pyun, Escherichia coli with autodisplayed Z-domain of protein A for signal amplification of SPR biosensor, Biosens. Bioelectron. 24 (2009) 1324–1329, https://doi.org/10.1016/j.bios.2008.07.067.
[32] G.Y. Lee, J.H. Park, Y.W. Chang, S. Cho, M.J. Kang, J.C. Pyun, Chronoamperometry-based redox cycling for application to immunoassays, ACS Sens. 3 (2018) 106–112, https://doi.org/10.1021/acssensors.7b00681.
[33] G.-Y.Y. Lee, J.-H.H. Park, Y.W. Chang, M.-J.J. Kang, S. Cho, J.-C.C. Pyun, Capacitive biosensor based on vertically paired electrode with controlled parasitic capacitance, Sensors Actuators, B Chem. 273 (2018) 384–392, https://doi.org/10.1016/j.snb.2018.06.050.
[34] R.M. McCormick, B.L. Karger, Guidelines for data acquisition and data quality evaluation in environmental chemistry, Anal. Chem. 52 (1980) 2242–2249, https://doi.org/10.1021/ac50064a004.
[35] J.E.B. Randles, Kinetics of rapid electrode reactions, Faraday Discuss. 1 (1947) 11–19, https://doi.org/10.1039/DF9470100011.